SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
BIOMATERIALS - CELLS
ISOLATION, SEPARATION, PURIFICATION

Received
26 February 2014

Accepted
2 April 2014

Published
7 May 2014

Correspondence and requests for materials should be addressed to H.M. (hmatsui@md.tsukuba.ac.jp)

Optical cell separation from three-dimensional environment in photodegradable hydrogels for pure culture techniques

Masato Tamura[1], Fumiki Yanagawa[2], Shinji Sugiura[2], Toshiyuki Takagi[2], Kimio Sumaru[2], Hirofumi Matsui[1] & Toshiyuki Kanamori[2]

[1]Faculty of Medicine, University of Tsukuba 1-1-1 Ten-noudai, Tsukuba, Ibaraki 305-8575, Japan, [2]Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Central 5th, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan.

Cell sorting is an essential and efficient experimental tool for the isolation and characterization of target cells. A three-dimensional environment is crucial in determining cell behavior and cell fate in biological analysis. Herein, we have applied photodegradable hydrogels to optical cell separation from a 3D environment using a computer-controlled light irradiation system. The hydrogel is composed of photocleavable tetra-arm polyethylene glycol and gelatin, which optimized cytocompatibility to adjust a composition of crosslinker and gelatin. Local light irradiation could degrade the hydrogel corresponding to the micropattern image designed on a laptop; minimum resolution of photodegradation was estimated at 20 μm. Light irradiation separated an encapsulated fluorescent microbead without any contamination of neighbor beads, even at multiple targets. Upon selective separation of target cells in the hydrogels, the separated cells have grown on another dish, resulting in pure culture. Cell encapsulation, light irradiation and degradation products exhibited negligible cytotoxicity in overall process.

Cell sorting is an essential and an efficient experimental tool for the isolation and characterization of a target cell population; it is widely used in basic cell biology and medical applications. Fluorescence-activated cell sorting (FACS) and magnetic cell sorting (MACS) have been commonly used to analyze large populations of suspended cells with biochemical characteristics of cell surface markers[1,2]. Microfabrication technology[3], microfluidic devices[4,5], and optical trapping[6] allow to manipulate small numbers of floating cells in suspension and to attach cells onto micropatterned two-dimensional (2D) surfaces. In biological analysis, a three-dimensional (3D) cultural environment is crucial in determining cell behavior and cell fate in tissue morphogenesis, stem cell differentiation, and cancer progression[7], because the 3D culture environment with soluble factors and extracellular matrix regulates cellular function and phenotype[8,9]. Thus, an efficient method to analyze and separate cellular populations in a 3D culture environment is requisite in biological studies.

Optical techniques are promising approach for cell micropatterning and micromanipulations. Light irradiation can control the object locally and instantly in a non-contact manner. For use this, synthetic photoresponsive materials have achieved cell micropatterning on the 2D surface coated with the modified arginine-glycine-aspartate cell-adhesive peptide[10–12], cell-adhesive proteins[13–15], and 2-methacryloyloxyethyl phosphorylcholine polymer[16–18]. For these cell micropatterning techniques, special equipment such as a photomask and mask aligner is required to irradiate micropatterned light. Such expensive or large pieces of equipment are not available in general biology laboratories. To address this issue, we developed a computer-controlled light irradiation system, in which a micropattern projection unit is equipped on a commercially available inverted microscope, and demonstrated step-wise micropatterning of multiple cells without using a photomask[19].

Photodegradable hydrogels have recently been developed to create 3D microstructures and to control the 3D microenvironment[20,21]. Such photodegradable hydrogels have garnered substantial attention from the biomaterials and tissue engineering research fields[22]. Degradation depth can be controlled by irradiated light energy[20,23], meanwhile, complicated 3D micropatterned degradation was demonstrated by two-photon light irradiation[24].

Physical and chemical properties of photodegradable hydrogels are temporally and spatially controllable by light exposure[23,24]. Of note, photodegradation is compatible with living cells[20,22].

We developed a photoresponsive culture surface with poly(*N*-isopropylacrylamide), which can control cell adhesion by light irradiation[25]. Light irradiation on these photoresponsive materials induced cleavage or isomerization of the photoresponsive materials, resulting in control of cell adhesion on the micrometer scale corresponding to the irradiated light pattern. Most recently, we synthesized a novel class of photocleavable crosslinker, which can form photodegradable hydrogels through a two-component mixing reaction with polymers containing amino moieties (e.g. gelatin) and demonstrated micropatterned degradation of hydrogels[26]. We used the photodegradable hydrogels as a scaffold, the cells can attach on the hydrogels and the hydrogels were successfully micropatterned[26]. In this study, we have applied this photodegradable hydrogel to optical cell separation from a 3D culture environment using a computer-controlled light irradiation system. We also optimized the encapsulation conditions, such as crosslinking density, and obtained a harmless condition for encapsulation and obtaining cells without any cell damage in terms of cell growth.

Results

Formation of photodegradable hydrogels and micropatterned degradation. Strategies of this study are shown in figure 1. The synthesized *N*-hydroxysuccinimide (NHS)-terminated photocleavable tetra-arm PEG (NHS-PC-4armPEG) crosslinker is composed of tetra-arm PEG with photocleavable *o*-nitrobenzyl groups and amine-reactive NHS-activated ester groups (Figure 1A)[26]. The photodegradable hydrogels were prepared by performing a crosslinking reaction between NHS moieties in the NHS-PC-4armPEG and amino moieties in gelatin, and degraded by light irradiation (Figure 1B). We encapsulated cells into photodegradable hydrogels composed of NHS-PC-4armPEG and gelatin, and picked the target cells by local degradation of the hydrogel induced by micropatterned light irradiation (Figure 1C).

Micropatterned degradation of the photodegradable hydrogel was performed using the computer-controlled light irradiation system for determining the resolution of photo-induced degradation (Figure 1D). The minimum resolution of degradation was estimated at 20 μm by the smallest circles formed in the hydrogel (Figure 1D, right). Degradation of the hydrogel was further proved, after staining with CBB, by the observation that the hydrogels encapsulated fluorescent microbeads (supporting information Figure S1). The CBB-stained hydrogel, indicated as blue color in the phase-contrast image, contained fluorescent microbeads, and no microbead was observed outside the hydrogels, showing complete degradation of the hydrogel in the irradiated area.

Optical microbeads separation. To evaluate preconditions for cell separation, we demonstrated optical microbeads separation in the photodegradable hydrogels. As shown in Figure 2 (supporting information Figure S7), the encapsulated microbeads were separated by micropatterned light irradiation using a computer-controlled light irradiation system. Upon using a large circular pattern of irradiation, the microbeads in the irradiated area were completely removed from the hydrogels (Figure 2A). Single microbeads of about 20 μm size were picked from the light irradiation area (Figure 2B). The selective choice of microbeads was performed by irradiating micropatterned light to the location of the target microbeads (Figure 2C). Multiple target microbeads (green) were selectively picked away, leaving most of non-target microbeads (red) intact in the hydrogel. These results indicated that the resolution met a requirement for cell separation.

Optical cell selection from 3D environment for pure culture. To establish pure culture from 3D environment, a target cell need to be separated from several kinds of cells in hydrogel. We demonstrated optical cell separation from the model co-culture system. For the easily identification of isolated cells, we targeted RGK-KO cells to demonstrate selective cell separation from the co-culture (Figure 3A and supporting information Figure S8). The target cells were identified by their red fluorescent image, and local light was irradiated on the target RGK-KO cells by the computer-controlled light irradiation system (365 nm, 263 mW/cm^2, 20 s). The isolated RGK-KO cells were retrieved from the photodegradable hydrogel and cultured in another culture dish. The retrieved RGK-KO grew well and no contamination was observed in the RGM cells in the collection dish (Figure 3A, right). The cell separation processes including light irradiation had not caused cell death.

The cytotoxic effect of the cell separation process. For an improvement of cytocompatibility of cell separation process, the cytotoxic effect of the cell encapsulation process was investigated in various conditions. We evaluated cell viability in hydrogels composed of different NHS-PC-4armPEG/gelatin weight ratios and gelatin concentrations (Figure 3B and Table 1). Cell viability was more than 70% in the hydrogels composed of 0.43, 0.87, and 1.30 NHS-PC-4armPEG/gelatin weight ratios and 1.25% (w/v) gelatin, and 0.43 NHS-PC-4armPEG/gelatin weight ratio and 2.5% (w/v) gelatin. Cells did not survive in the hydrogel with NHS-PC-4armPEG/gelatin weight ratios greater than 0.43 and 2.5% (w/v) gelatin, and all NHS-PC-4armPEG/gelatin weight ratio and 5.0% (w/v) gelatin. For clarifying the cytotoxic effect of NHS-PC-4armPEG, NHS-PC-4armPEG was added to the cell co-culture medium in the adhesion culture on the culture dishes (Figure 4). The cell viability after 30 min incubation in the medium containing 1.0% (w/v) NHS-PC-4armPEG was 48.6%.

We also investigated the potential cytotoxic effect induced by the degradation products of the photodegradable hydrogel. The retrieved photodegradation products of the hydrogel were added to the co-culture medium in the adhesion culture on the culture dishes (Figure 5). The degradation products exhibited a cytotoxic effect in a concentration-dependent manner at concentrations greater than 0.3% (v/v). Finally, we examined that potential cytotoxic effects induced by light irradiation (Figure 6). Light irradiation at less than 42 J/cm^2 did not cause cytotoxicity.

Discussion

Our strategy to separate the target cells in photodegradable cells includes; (1) encapsulation of cells in photodegradable hydrogels and (2) cell separation by light irradiation (Figure 1C). In this study, we have performed these processes with low cytotoxicity.

In the cell encapsulation process, cell viability decreased with increasing concentrations of NHS-PC-4armPEG (Figure 3B). The photodegradable hydrogels that exhibited cell viability greater than 70% (Figure 3B) contained up to 1.63% (w/v) of NHS-PC-4armPEG (Table 1). The increase in cell viability was indicated in the hydrogel rather than in the solution, although both systems contained a similar amount of NHS-PC-4armPEG. According to these results, the hydrogel with a composition of 0.43 NHS-PC-4armPEG/gelatin weight ratio of 1.25% (w/v) gelatin was found to be appropriate for cell selection studies. These results indicate that cytotoxicity caused by NHS moieties can be reduced by gelatin, which contains many amino moieties that may react with NHS moieties, while the cell viability in photodegradable hydrogels decreased even with an increasing concentration of NHS-PC-4armPEG. Although we found cell encapsulation condition to maintain as high cell viability as 70%, it should be difficult to completely avoid the toxic effect of the NHS moiety in the strategy presented in this study. The previous study reported the cupper-free click reaction[21], which could resolve the problem of the NHS moiety.

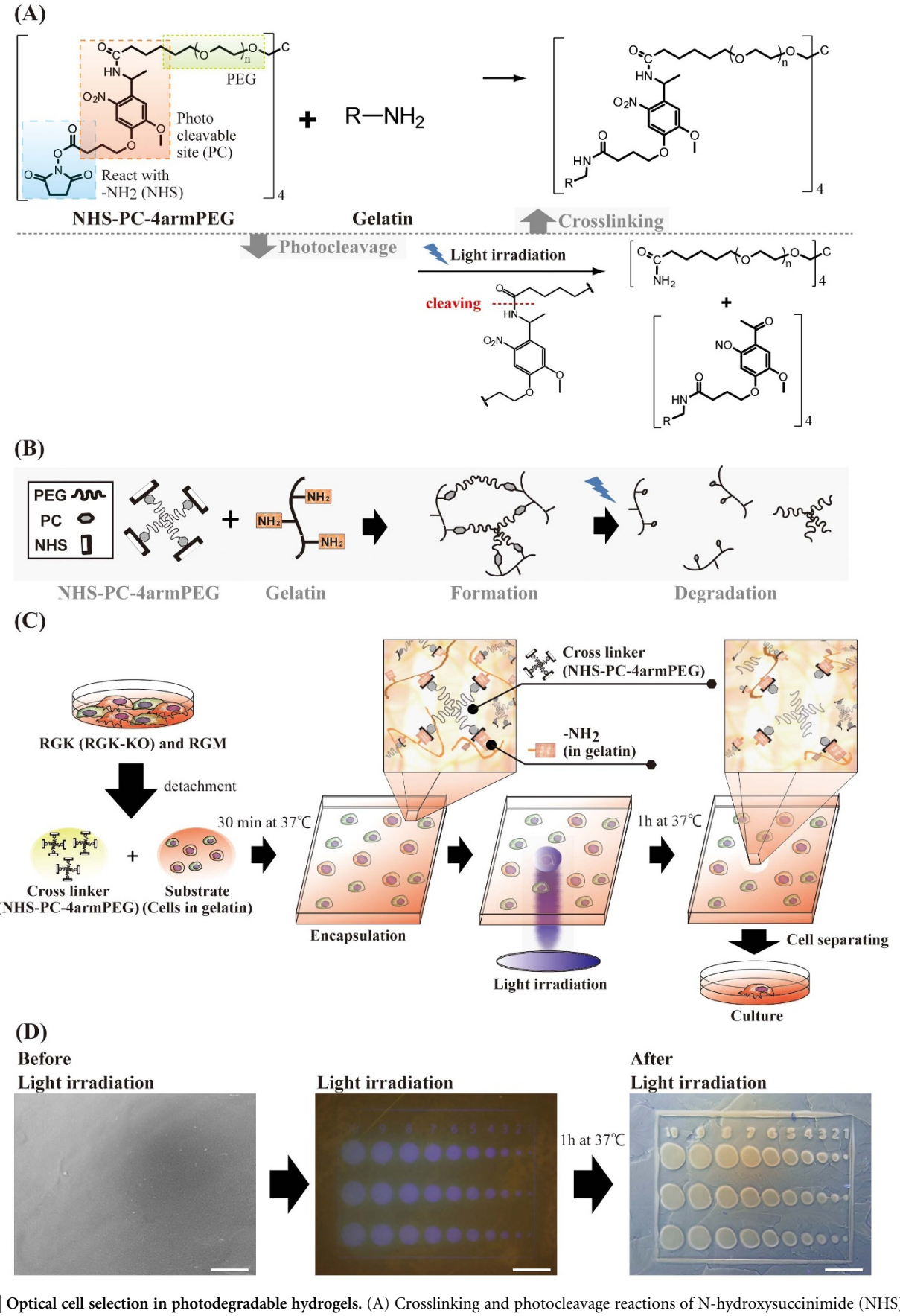

Figure 1 | Optical cell selection in photodegradable hydrogels. (A) Crosslinking and photocleavage reactions of N-hydroxysuccinimide (NHS)-terminated photocleavable tetra-arm polyethylene glycol (NHS-PC-4armPEG). (B) Formation and photo-induced degradation of the photodegradable hydrogels. (C) Cell encapsulation in photodegradable hydrogel and optical cell selection. (D) Micropatterned degradation of the photodegradable hydrogel. Scale bar: 250 μm.

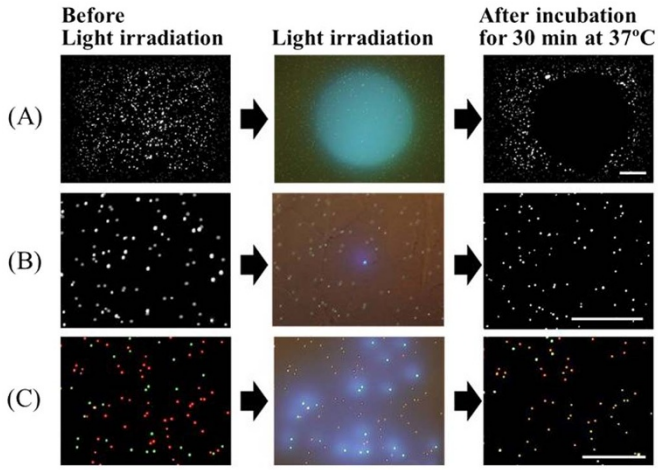

Figure 2 | Optical microbeads selection in the photodegradable hydrogels. Selective picking of microbeads was demonstrated by light irradiation on (A) a large circle area, (B) a single microbead, and (C) multiple microbeads. Scale bars: 250 μm.

Cancer cells exhibit different properties compared to normal cells. For example, RGM cannot invade into matrigel, however, RGK cells can[27]. This difference can be used to distinguish cancer cells from normal cells in the hydrogels composed of the biomimetic material capable of reproducing this property. The hydrogel with a composition of 0.43 NHS-PC-4armPEG/gelatin weight ratio and 1.25% (w/v) gelatin showed this property; RGM could not invade within the hydrogel after incubation for 96 h, however RGK could (supporting information Figure S2). Of course, both of the cells were alive in the hydrogel after 96 h (supporting information Figure S3). These results indicate that the hydrogels mimic the condition of matrigel in cellular invasion.

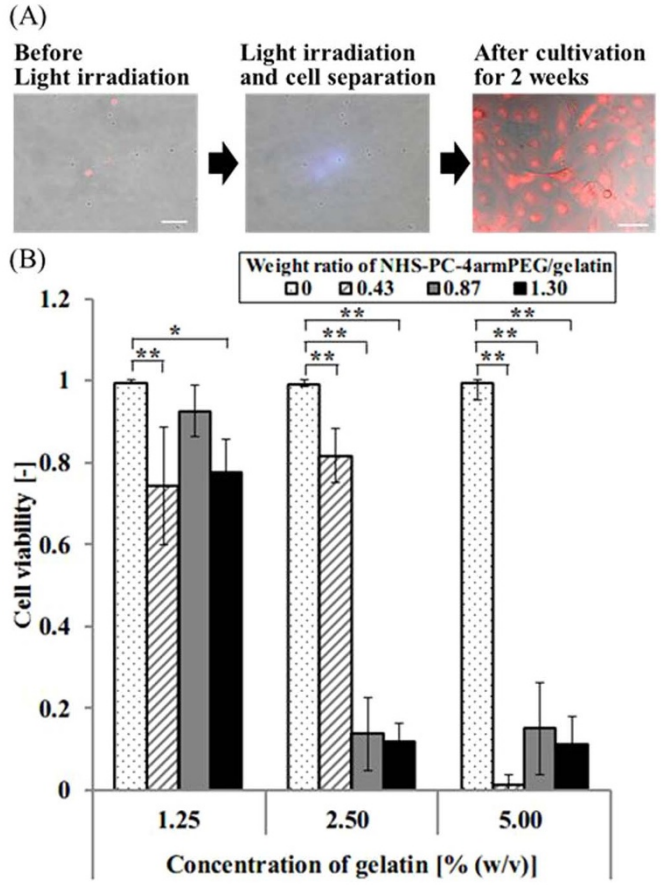

Figure 3 | Optical cell selection in photodegradable hydrogels. (A) Selective cell separating from co-culture and cultivation of the isolated cells. The isolated RGK-KO cells were cultured on another dish for 2 weeks. Scale bars: 100 μm. (B) Cell viability test by LIVE DEAD assay. Statistically significant differences are denoted as *$p < 0.05$ and **$p < 0.01$ ($n = 4$).

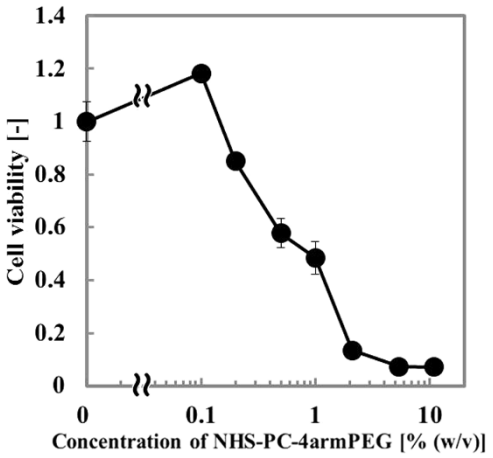

Figure 4 | Cytotoxicity of NHS-PC-4armPEG in adhesion culture evaluated by WST assay. RGK and RGM cells were inoculated in a 96-well plate at the total cell density of 1×10^4 cells/well [mixing ratio of RGK and RGM was 1:1 (n/n)]. After adhesion culture for 24 h, NHS-PC-4armPEG was added to the medium and the cells were incubated at 37°C for 30 min. Cell viability was calculated relative to the untreated cells. Error bars indicate standard deviation ($n = 6$).

In the cell separation process, exposure of the degradation products and light irradiation were considered to be the cause of cytotoxicity. In fact, degradation products induced a completely cell death at the concentration range higher than 1.2% (v/v) of degradation products (Figure 5). However, we believe that the cytotoxic effect induced by degradation products does not cause any problem during optical cell picking process because the

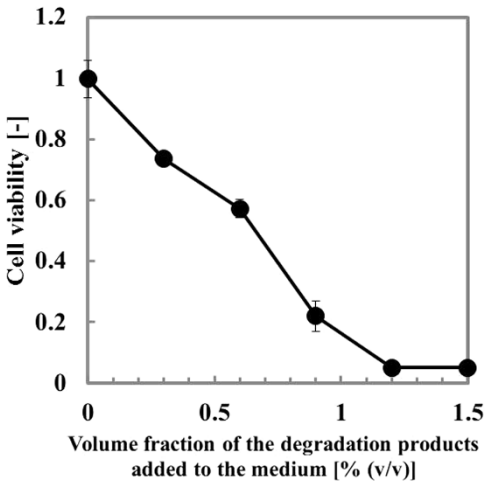

Figure 5 | Cytotoxicity of the degradation products of photodegradable hydrogel evaluated by the WST assay. The degradation products were generated from 30 μL of the photodegradable hydrogels composed of 1.09% (w/v) NHS-PC-4armPEG and 2.5% (w/v) gelatin (0.43 weight ratio of NHS-PC-4armPEG/gelatin). Cell viability was determined by the WST assay after incubation with the degradation products for 12 h. Error bars indicate standard deviation ($n = 5$).

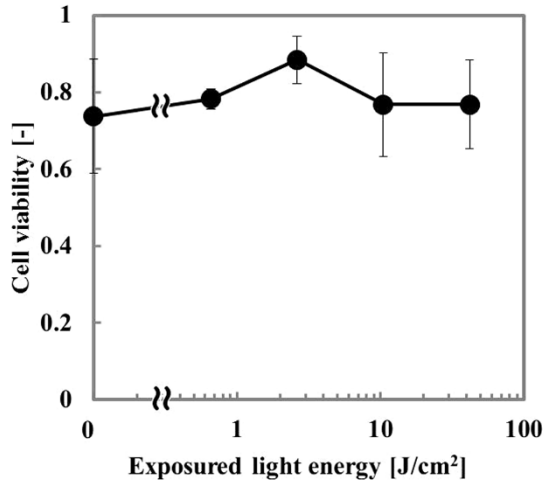

Figure 6 | Cytotoxicity of light irradiation evaluated by LIVE DEAD assay. The cell viability was determined by LIVE DEAD assay following light irradiation (365 nm, 29.9 mW/cm^2, 20 s). The number of counted cells for each data point was more than 100. Error bars indicate standard deviation (n = 3).

volume of the degraded hydrogel was little. The actual volume of the degraded hydrogel was estimated to be between 7.85 pL to 20 nL for the degradation of hydrogels with 20 μm to 1 mm diameters and 25-μm thickness. This volume was less than 0.001% of the medium in the culture dish. From these considerations, we concluded that the cytotoxic effect induced by degradation products could be negligible in our separation condition. We also investigated potential cytotoxic effects induced by light irradiation (Figure 6). We did not observe any effect on the cell viability of RGK and RGM by light irradiation by 8-times stronger exposure (42 J/cm^2) than that we used in the optical cell picking process (5.2 J/cm^2). It is reported that a high dose of UV light irradiation (more than 500 J/cm^2) induced DNA damage in cells and inhibited cell growth[28], whereas the UV sensitivity depends on the cell line type[29]. Further investigation on the effect of light irradiation is necessary for the specific application of optical cell picking; for example, a colony-forming assay can be performed to determine DNA damages. Despite the potential cytotoxic effect induced by degradation products and light irradiation, the optimized conditions demonstrated in Figure 3A did not induce severe cell damage by NHS-PC-4armPEG, degradation products, or light irradiation, as evidenced in Figures 3B and Figure 4, Figure 5, and Figure 6, respectively.

Cell separation and sorting are essential steps in cell biology research to reduce heterogeneity in studies on cell samples such as stem cells, circulating tumor cells, and cancer stem cells. Living cells exhibit their function in a 3D environment. In hydrogels, cells respond to chemical composition as well as to the stiffness of hydrogels, and these parameters then affect cell viability and phenotypes[30–33]. Therefore, tuning the physicochemical properties of the hydrogel is important for versatile application of optical cell selection. The crosslinker used in this study can form photodegradable hydrogels through a reaction with a variety of polymers containing amino moieties[26]. The chemical composition and stiffness of the hydrogels can also be controlled by changing the composition of the crosslinker and polymers. For practical application of our optical cell separation to a specific cell type, further optimization is necessary to determine the appropriate chemical composition and stiffness of hydrogels in order to allow encapsulated cells exhibiting preferable function and phenotype in a 3D culture environment with maintaining the ability of photodegradation.

Methods

Materials. N-hydroxysuccinimide (NHS)-terminated photocleavable tetra-arm PEG (NHS-PC-4armPEG) crosslinker, which is composed of 4-arm PEG with photocleavable o-nitrobenzyl groups and amine-reactive NHS-activated ester groups, was synthesized according to previously described method (Supporting information Figure S4)[34]. 3-(1-Piperazino)-propyl-functionalized silica gel was washed with dimethyl sulfoxide (DMSO) before use. 4-[4-[1-(Fmoc)ethyl]-2-methoxy-5-nitrophenoxy]butanoic acid (2.7 g; Advanced Chemtech, Louisville, KY, USA) was added to 3-(1-piperazino)propyl functionalized silica gels in DMSO (66 g/130 mL) for 24 h. After the insoluble silica gel was removed by filtration, the volume of the resulting unprotected amino compound in DMSO was decreased to about 130 mL by reduced-pressure distillation. Purified reactants were added to pentaerythritol tetra succinimidyl carboxypentyl polyoxyethylene (9.6 g; NOF Co., Tokyo, Japan) solutions in tetrahydrofuran (THF), and then stirred at room temperature for about 24 h. To the THF solution of the carboxylic acid compound, N-hydroxysuccinimide (NHS, Tokyo Chemical Ind. Co., Tokyo, Japan) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, Tokyo Chemical Ind. Co.) were added, and the mixture was stirred for about 24 h, followed by purifications by ether precipitation, drop-wise addition into ether at 0°C, and collection by filtration. After two more precipitations by ether, the precipitate was purified with Shephadex LH-20 in methanol. The solvents were replaced to dichloromethane and the dichloromethanephase was washed by mixing 5% hydrochloric acid solution and brine. The phase was dried over magnesium sulfate anhydride, filtered, and concentrated. After three further purifications by ether precipitation, 9.8 g of NHS-PC-4armPEG was obtained (Supporting information Figure S5). Gelatin (Sigma-Aldrich Co. LLC., St. Louis, MO), Quick-CBB PLUS (Wako Pure Chemical Ind., Ltd.), fluorescent microbeads (green fluorescent microbead, 10 μm, Duke Scientific Co., Palo Alto, CA; red fluorescent microbead, 3 μm, Duke Scientific Co.), LIVE DEAD reagent (Life technologies) and WST reagent (Dojindo, Kumamoto, Japan) were purchased and used without further purification.

Cell culture. RGK[35] and RGM[36] were established in our previous studies. RGK and RGM were cultured in Dulbecco's modified eagle's medium nutrient mixture F-12 HAM (Life technologies, Carlsbad, CA) and DMEM/F12 (Life technologies), respectively, in a 5% CO2 humidified atmosphere at 37°C. This medium contained 10% inactivated fetal bovine serum (FBS, Life technologies), 1% penicillin/streptomycin, and 0.2 mg/mL G418 sulfate (Life technologies), for inhibition the growth from Kusabira orange (red fluorescence)-expressing RGK (RGK-KO) without fluorescence protein-expressing vector. In the cell-selection study, separated cells were cultured in the co-culture medium, the half-volume mixture medium contained both the medium for RGK and RGM without G418 sulfate.

Formation of photodegradable hydrogels and encapsulation of microbeads and cells. The crosslinker solution was prepared by dissolving NHS-PC-4armPEG in 10 mM phthalic acid (Wako Pure Chemical Ind., Osaka, Japan) and 140 mM sodium chloride aqueous solution (pH 4.0). Gelatin solution was prepared by dissolving gelatin in a mixture solution of equal volume of 300 mM HEPES (pH 7.0) and culture medium without FBS and penicillin/streptomycin at 37°C for 10 min. After mixing 15 μL of the crosslinker solution with an equal volume of the gelatin solution, the mixture was casted on a 35-mm lysine-coated culture dish (AGC Techno Glass Co., Ltd., Shizuoka, Japan) and was covered with poly(dimethylsiloxane) block (0.5 cm × 1 cm × 1 cm) using 25-μm-thick polytetrafluoroethylene films as spacers. After incubation at 37°C for 30 min, the hydrogel was washed twice with the co-culture medium. For microbeads encapsulation, the gelatin solution was prepared with fluorescent microbeads (green fluorescent microbead, 10 μm; red fluorescent microbead, 3 μm) at the density of 2 × 10^6 microbeads/mL, containing an equal number of green and red fluorescent microbeads. For cell encapsulation, the gelatin solution was prepared with RGK-KO and RGM cells at the total cell density of 2 × 10^6 cells/mL, containing an equal number of each cell. To encapsulate the cells in the gelatin solution, the cells were harvested using 1% trypsin solution and resuspended in the gelatin solution. The hydrogels containing microbeads and stained cells were formed according to the abovementioned protocol.

Micropatterned degradation studies. A photodegradable hydrogel composed of 0.54% (w/v) NHS-PC-4armPEG and 1.25% (w/v) gelatin (0.43 weight ratio of NHS-PC-4armPEG/gelatin) was used for all degradation studies. Micropatterned images were designed as bitmap images on a laptop. To degrade the hydrogels, micropatterned light was irradiated on the hydrogels (365 nm, 263 mW/cm^2, 20 s) using a computer-controlled light irradiation system, which is capable of maskless light irradiation of the designed micropatterned images[19]. The irradiated hydrogels were immersed in the co-culture medium and incubated at 37°C for 1 h to allow the degraded polymers to erode. Hydrogel micropatterning and cell selection studies were performed with the same procedure. In the hydrogel micropatterning study, the hydrogel micropatterns were visualized by CBB staining. After washing the hydrogels with PBS twice, the hydrogels were immersed in the Quick-CBB PLUS and incubated at room temperature for 1 h. In microbeads or cell picking studies, the irradiated hydrogels were immersed in the co-culture medium and incubated at 37°C for 30 min. These hydrogels were observed after washing with PBS. The isolated cells were transferred to another culture dish and incubated in a 5% CO2 humidified atmosphere at 37°C for 2 weeks.

Cell viability test by LIVE DEAD assay. The 1 : 1 mixture of RGK and RGM cells was used for cell viability test. LIVE DEAD assay was performed according to the manufacture's instruction. Briefly, the hydrogel containing cells was incubated with LIVE DEAD reagent, 1 μL of calcein AM (live) with 4 μL of ethidium homodimer-1 (dead) in 5 mL PBS at 37°C for 30 min. After washing the hydrogel twice with PBS, pictures were taken with a confocal laser microscopy (LSM 700, Zeiss, Thornwood, NY). Cell viability was determined from the live/dead images. More than 100 cells were counted for each data point. During quantification, the green fluorescent cells were ascribed to live cells and cells with both green and red fluorescent were ascribed to dead cells. Cell viability in each experimental condition was normalized by that in the gelatin solution without NHS-PC-4armPEG.

Cell viability test by WST assay. WST assay was performed according to the manufacture's instruction and previous report[37]. Briefly, RGK and RGM cells were inoculated in a 96-well plate at the total cell density of 1×10^4 cells/well [mixing ratio of RGK and RGM was 1 : 1 (n/n)]. After adhesion culture for 24 h, chemicals were added to the medium and the cells were incubated at 37°C for 30 min. Cell viability was determined by the WST assay. The medium was replaced to 10 v% WST reagent-contained medium and incubated at 37°C for 1 h. Then, the absorbance at 450 nm of the formazan produced by living cells was measured using a microplate reader (Varioskan, Thermo Electron Co., Waltham, MA). Cell viability was calculated relative to the untreated cells.

Statistical analysis. Data were statistically analyzed using two-way analysis of variance (ANOVA) and Bonferroni's post-hoc test by using the Kaleida Graph software (Hulinks Inc., Tokyo, Japan).

1. Dainiak, M. B., Kumar, A., Galaev, I. Y. & Mattiasson, B. Methods in cell separations. *Adv. Biochem. Eng. Biotechnol.* **106**, 1–18 (2007).
2. Browne, S. M. & Al-Rubeai, M. Selection methods for high-producing mammalian cell lines. *Trends Biotechnol.* **25**, 425–32 (2007).
3. Di Carlo, D. & Lee, L. P. Dynamic single-cell analysis for quantitative biology. *Anal. Chem.* **78**, 7918–25 (2006).
4. Wheeler, A. R. *et al*. Microfluidic device for single-cell analysis. *Anal. Chem.* **75**, 3581–6 (2003).
5. Mu, X., Zheng, W., Sun, J., Zhang, W. & Jiang, X. Microfluidics for manipulating cells. *Small* **9**, 9–21 (2013).
6. Hosokawa, Y. *et al*. Nondestructive isolation of single cultured animal cells by femtosecond laser-induced shockwave. *Appl. Phys. A* **79**, 795–798 (2004).
7. Martin, I., Wendt, D. & Heberer, M. The role of bioreactors in tissue engineering. *Trends Biotechnol.* **22**, 80–6 (2004).
8. Cukierman, E., Pankov, R., Stevens, D. R. & Yamada, K. M. Taking cell-matrix adhesions to the third dimension. *Science* **294**, 1708–12 (2001).
9. Pampaloni, F., Reynaud, E. G. & Stelzer, E. H. K. The third dimension bridges the gap between cell culture and live tissue. *Nat. Rev. Mol. Cell Biol.* **8**, 839–45 (2007).
10. Ohmuro-Matsuyama, Y. & Tatsu, Y. Photocontrolled cell adhesion on a surface functionalized with a caged arginine-glycine-aspartate peptide. *Angew. Chem. Int. Ed. Engl.* **47**, 7527–9 (2008).
11. Petersen, S. *et al*. Phototriggering of cell adhesion by caged cyclic RGD peptides. *Angew. Chem. Int. Ed. Engl.* **47**, 3192–5 (2008).
12. Liu, D., Xie, Y., Shao, H. & Jiang, X. Using azobenzene-embedded self-assembled monolayers to photochemically control cell adhesion reversibly. *Angew. Chem. Int. Ed. Engl.* **48**, 4406–8 (2009).
13. Nakanishi, J. *et al*. Spatiotemporal control of migration of single cells on a photoactivatable cell microarray. *J. Am. Chem. Soc.* **129**, 6694–5 (2007).
14. Kaneko, S. *et al*. Photocontrol of cell adhesion on amino-bearing surfaces by reversible conjugation of poly(ethylene glycol) via a photocleavable linker. *Phys. Chem. Chem. Phys.* **13**, 4051–9 (2011).
15. Guo, Q. *et al*. Light activated cell migration in synthetic extracellular matrices. *Biomaterials* **33**, 8040–6 (2012).
16. Jang, K. *et al*. Surface modification by 2-methacryloyloxyethyl phosphorylcholine coupled to a photolabile linker for cell micropatterning. *Biomaterials* **30**, 1413–20 (2009).
17. Tomatsu, I., Peng, K. & Kros, A. Photoresponsive hydrogels for biomedical applications. *Adv. Drug Deliv. Rev.* **63**, 1257–66 (2011).
18. Goubko, C. A., Basak, A., Majumdar, S. & Cao, X. Dynamic cell patterning of photoresponsive hyaluronic acid hydrogels. *J. Biomed. Mater. Res. A* **102**, 381–91 (2014).
19. Kikuchi, K. *et al*. Stepwise assembly of micropatterned co-cultures using photoresponsive culture surfaces and its application to hepatic tissue arrays. *Biotechnol. Bioeng.* **103**, 552–61 (2009).
20. Kloxin, A. M., Kasko, A. M., Salinas, C. N. & Anseth, K. S. Photodegradable hydrogels for dynamic tuning of physical and chemical properties. *Science* **324**, 59–63 (2009).
21. DeForest, C. A. & Anseth, K. S. Cytocompatible click-based hydrogels with dynamically tunable properties through orthogonal photoconjugation and photocleavage reactions. *Nat. Chem.* **3**, 925–31 (2011).
22. Lutolf, M. P. Materials science: Cell environments programmed with light. *Nature* **482**, 477–8 (2012).
23. Kloxin, A. M., Tibbitt, M. W., Kasko, A. M., Fairbairn, J. A. & Anseth, K. S. Tunable hydrogels for external manipulation of cellular microenvironments through controlled photodegradation. *Adv. Mater.* **22**, 61–6 (2010).
24. Wong, D. Y., Griffin, D. R., Reed, J. & Kasko, A. M. Photodegradable Hydrogels to Generate Positive and Negative Features over Multiple Length Scales. *Macromolecules* **43**, 2824–2831 (2010).
25. Edahiro, J.-I. *et al*. In situ control of cell adhesion using photoresponsive culture surface. *Biomacromolecules* **6**, 970–4 (2005).
26. Yanagawa, F., Sugiura, S., Takagi, T., Sumaru, K. & Kanamori, T. Photo-cleavable crosslinker capable of preparing photodegradable hydrogel by a two component reaction for hydrogel micro patterning. *Proc. mTAS* 149–151 (2013).
27. Tamura, M. *et al*. Mitochondrial reactive oxygen species accelerate gastric cancer cell invasion. *J. Clin. Biochem. Nutr.* **54**, 12–17 (2013).
28. Callegari, A. J. & Kelly, T. J. Shedding light on the DNA damage checkpoint. *Cell Cycle* **6**, 660–6 (2007).
29. Itoh, T. Xeroderma pigmentosum group E and DDB2, a smaller subunit of damage-specific DNA binding protein: proposed classification of xeroderma pigmentosum, Cockayne syndrome, and ultraviolet-sensitive syndrome. *J. Dermatol. Sci.* **41**, 87–96 (2006).
30. Zorlutuna, P. *et al*. Microfabricated biomaterials for engineering 3D tissues. *Adv. Mater.* **24**, 1782–804 (2012).
31. Khetan, S. & Burdick, J. A. Patterning hydrogels in three dimensions towards controlling cellular interactions. *Soft Matter* **7**, 830 (2011).
32. Cha, C., Jeong, J. H., Shim, J. & Kong, H. Tuning the dependency between stiffness and permeability of a cell encapsulating hydrogel with hydrophilic pendant chains. *Acta Biomater.* **7**, 3719–28 (2011).
33. Cha, C., Kim, S. Y., Cao, L. & Kong, H. Decoupled control of stiffness and permeability with a cell-encapsulating poly(ethylene glycol) dimethacrylate hydrogel. *Biomaterials* **31**, 4864–71 (2010).
34. Yanagawa, F. *et al*. Activated-ester-type photocleavable crosslinker for preparation of photodegradable hydrogels by a two-component mixing reaction. *Chem. Mater.* Submitted (2013).
35. Shimokawa, O. *et al*. Neoplastic transformation and induction of H+, K+ -adenosine triphosphatase by N-methyl-N'-nitro-N-nitrosoguanidine in the gastric epithelial RGM-1 cell line. *In Vitro Cell. Dev. Biol. Anim.* **44**, 26–30 (2008).
36. Kobayashi, I. *et al*. RGM1, a cell line derived from normal gastric mucosa of rat. *In Vitro Cell. Dev. Biol. Anim.* **32**, 259–61 (1996).
37. Tamura, M., Ichinohe, S., Tamura, A., Ikeda, Y. & Nagasaki, Y. In vitro and in vivo characteristics of core-shell type nanogel particles: optimization of core cross-linking density and surface poly(ethylene glycol) density in PEGylated nanogels. *Acta Biomater.* **7**, 3354–61 (2011).

Acknowledgments

This work was partially supported by the Japan Society for the Promotion of Science (JSPS) and Grant-in-Aid for Scientific Research (KAKENHI) #24106503, #24106512.

Author contributions

M.T. and F.Y. contributed equally to this work. M.T., F.Y., S.S., T.T., K.S., H.M. and T.K. were involved in the design of experiments. K.S. helped in light irradiation using a computer-controlled light irradiation system. T.T. prepared photocleavable tetra-arm polyethylene glycol and supporting figures S4 and S5. M.T. and S.S. wrote the manuscript. All authors discussed the results and commented on the manuscript.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Tamura, M. *et al*. Optical cell separation from three-dimensional environment in photodegradable hydrogels for pure culture techniques. *Sci. Rep.* **4**, 4793; DOI:10.1038/srep04793 (2014).